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Applicant: Martin et al.

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF MICHAEL S. NEUBERGER, Ph.D.

I, Michael S. Neuberger, do hereby declare:

1. I am the Joint Head of the Division of Protein and Nucleic Acid Chemistry and a member of the Executive Committee of the Medical Research Council Laboratory of Molecular Biology in Cambridge. I am a member of the Scientific Advisory Board of AnaptysBio, Inc., which is a licensee of the present application, as well as a stockholder in AnaptysBio, Inc. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. In 2001 (as well as well before), somatic hypermutation (SHM) was known to involve a programmed process of mutation of variable regions of rearranged immunoglobulin genes that creates additional diversity within an expanding clone of B cells responding to an antigen. Specifically, following antigen recognition by B cells, a B cell enters the germinal center of peripheral lymphoid organs to become a centroblast B cell. In the germinal center, SHM occurs at rates of 10^{-5} to 10^{-3} mutations per base pair per generation, which is ~ 1 million-fold higher than the spontaneous rate of mutation in most other genes. Generally, the mutations are single base substitutions, with occasional insertions and deletions. While mutations occur throughout the rearranged V regions, the mutations are preferentially targeted to "hot spots" having the sequence WRCY (W=A or T, R=A or G, and Y=T or C) or WA motifs. Transition mutations arise more frequently than transversion mutations (see, e.g., Diaz et al., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 356(1405): 67-72 (2001)).

3. In 2001 (as well as well before), class switch recombination ("CSR") (also known as isotype switching) was known to be a mechanism by which the isotype (or class) of an antibody is changed (e.g., from IgM to IgG). During CSR, a portion of the antibody heavy chain locus is removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces an antibody of a different isotype. Double-stranded breaks are generated in DNA in or around conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY (2001)).

4. Although by 2001 it was well known that SHM and class switch recombination can occur in B cells at a similar stage of differentiation, it was also known at this time that SHM and CSR appeared to be very different and distinct biochemical processes and frequently occurred independently of each other. The evidence that SHM and CSR are independent processes was further supported by experiments showing that different molecules were involved in SHM and CSR. For example, as early as 2001, CSR was found to be perturbed by a deficiency in the enzyme DNA-PK_{CS}, whilst SHM was unaffected (see, e.g., Bemark et al., *J. Exp. Med.*, *192*(10): 1509-1514 (2000)). However, several molecules were suggested to be play some (albeit undefined) role in allowing both SHM and CSR, including activation-induced cytidine deaminase (AID) (see, e.g., Murumatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999), and Murumatsu et al., *Cell*, *102*: 553-563 (2000)), mismatch repair (MMR) proteins (see, e.g., Poltoratsky et al, *J. Exp. Med.*, *192*: F27-F30 (2000)), and error-prone DNA polymerases (see, e.g., Poltoratsky et al., *supra*).

5. In 2001 (as well as before), it was hypothesized that SHM was the result of a perturbation in the DNA polymerases involved in DNA damage repair, rather than an active DNA damage *per se* (see, e.g., Zeng et al., *Nat. Immunol.*, *2*: 537-541 (2001), Rogozin et al., *Nat. Immunol.*, *2*: 530-536 (2001), and Zan et al., *Immunity*, *14*: 643-653 (2001)).

6. By 2001, the main information known regarding the possible molecular function of AID came from its homology to the RNA-editing enzyme APOBEC1 as well as

from its purported deaminase activity on free cytidine (Muramatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999)),.

7. Murumatsu et al., *Cell*, *102*: 553-563 (2000) ("the Murumatsu reference"), which is cited in the Office Action, discloses that overexpression of AID in a lymphoma cell line augments antibody class switching from IgM to IgA without cytokine stimulation. The Muramatsu reference also discloses the generation of AID-deficient mice. AID deficiency completely blocked CSR in B cells activated by lipopolysaccharide (LPS) *in vitro* and by antigens *in vivo*. In addition, the Muramatsu reference demonstrates that B cells isolated from AID-deficient mice have failed to undergo the process of immunoglobulin gene somatic hypermutation. Based on these results, the Muramatsu reference hypothesizes that AID is an RNA editing enzyme that requires a co-factor for its activity (Muramatsu reference at page 560, first column, and page 561, first column).

8. The disclosure of the Muramatsu reference demonstrates that the AID gene is necessary for somatic hypermutation and class switch recombination, but the Murumatsu reference does not disclose the molecular function of AID or demonstrate that AID is actually involved in the mechanics of SHM and CSR. As discussed above, deficiencies in several other genes had previously been shown to affect somatic hypermutation and/or class switch recombination. In addition, the conclusions drawn by the Murumatsu reference with respect to the potential role of AID in SHM are based solely on the phenotype of AID knock-out mice. While the Murumatsu reference describes experiments in which AID is overexpressed in mouse B cells, it reports only on the effects of AID overexpression on CSR and not on SHM.

9. Clearly, the disclosure that a specific gene is necessary for a particular process is not at all the converse of showing that overexpression of the corresponding gene product is sufficient to induce the same process. In fact, that is very rarely the case. Thus, for example, many genes are necessary for the development of B lymphocytes as evidenced by B cell deficiency in a variety of mouse strains lacking specific transcription factors or cell surface molecules. However, in extremely few cases (if any) is ectopic expression of one of these gene products sufficient to induce B cell development in normal non-B-lineage cells. So the demonstration that a functional AID gene was necessary in order to allow CSR and SHM in

no sense indicated or even suggested that ectopic expression of AID would be sufficient to induce SHM. That was a striking and unanticipated discovery.

10. Thus, as of 2001, AID was one of many factors suspected to be involved in SHM and class switch recombination, but was not reported as the key enzyme responsible for induction of SHM. Moreover, at this time, the mechanism of action of activation-induced cytidine deaminase (AID) was entirely unknown (see, e.g., the Murumatsu reference and also Jacobs and Bross *Curr. Opin. Immunol.*, *13*(2): 208-218 (2001)). Indeed, Poltoratsky et al., *supra*, states that the mechanism of action of AID was unclear in 2000, and that V region mutation still occurs in mice and humans with defects in AID.

11. In 2001, it was not known nor suggested that AID alone is sufficient to induce SHM. In fact, such an idea would have been greeted with significant skepticism because of the complexity of SHM and class switch recombination processes, the essential restriction of these processes to the Ig locus, and the many other factors thought necessary to enable somatic hypermutation and class switch recombination *in vivo*, as described above. Indeed, one of ordinary skill in the art would not have considered that AID alone would be sufficient to induce SHM.

12. The Applicants' discovery that AID expression alone is sufficient to initiate somatic hypermutation, which is the foundation of the claimed invention, represented a significant shift in the thinking in the art. Specifically, this discovery prompted many of those of ordinary skill in the art to consider AID a key protein involved in mechanics of SHM.

13. In view of the foregoing, as of 2001 there was no reason to believe that AID directly induces the mutations in DNA that lead to SHM and class switch recombination. Moreover, there was no reason to believe that AID is capable of selectively targeting mutation of DNA sequences within the Ig locus. In addition, one usually could not accurately predict the molecular function of a particular protein based solely on the phenotype produced when the gene encoding the protein is disrupted or deleted.

14. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 2 November 2009 Michael N[illegible]

Exhibit A

CURRICULUM VITAE

Michael Samuel NEUBERGER PhD FRS

Work:
Medical Research Council
Laboratory of Molecular Biology
Division of Protein & Nucleic Acid Chemistry
Hills Road
Cambridge CB2 2QH

Tel: +44 (0) 1223 402481
Fax: +44 (0) 1223 412178
E-mail msn@mrc-lmb.cam.ac.uk

Home:
10 Maids Causeway
Cambridge
CB5 8DA

Tel: +44 (0) 1223 462100

Born: 2 November 1953

CAREER

1974:	BA University of Cambridge. First class honours in Natural Sciences (Biochemistry)
1974-1977:	Ph.D. Student under Professor B.S. Hartley at Department of Biochemistry, Imperial College, University of London.
1977:	Elected to a Research Fellowship at Trinity College, Cambridge.
1978:	Ph.D. (University of London). Thesis entitled "Transducing phages for analysis of gene duplications".
1977-1979:	Science Research Council Postdoctoral Fellow with Professor B.S. Hartley at Imperial College, London.
1979-1980:	EMBO Long-term Fellow with Professor K Rajewsky at the Institute of Genetics, University of Cologne.
1980-1985:	Medical Research Council short-term non-clinical staff appointment at the Laboratory of Molecular Biology, Cambridge.
1985 -	Permanent Scientific Staff appointment at the Medical Research Council Laboratory of Molecular Biology
1985-	Lecturer, Teaching Fellow and Director of Studies in Cell Biology and Biochemistry at Trinity College, University of Cambridge.
2002 -	Joint Head of Division of Protein and Nucleic Acid Chemistry and member of Executive Committee, Medical Research Council, Laboratory of Molecular Biology

HONOURS, KEYNOTES AND NAMED LECTURES

1986:	Max Perutz Prize at MRC Laboratory of Molecular Biology.
1989:	Member of European Molecular Biology Organisation
1992:	International Research Scholar of the Howard Hughes Medical Institute
1993:	Fellow of the Royal Society
2000:	Founder Fellow of the Academy of Medical Sciences
2000:	Royal College of Pathologists Kohn Lecturer
2001:	Novartis Medal (Biochemical Society)
2001:	William Bate Hardy Prize (Cambridge Philosophical Society)
2002:	Honorary Professor of Molecular Immunology, University of Cambridge
2002:	Sidney Leskowitz Memorial Lecturer (Tufts University)
2002:	Jean-Pierre Lecocq Prize (Academie des Sciences, Institut de France, France)
2002:	Keynote Speaker, First International Congress on Recombinant Antibodies, Berlin
2003:	GlaxoSmithKline Medal and Lecture (Royal Society)
2003:	Dannie-Heineman Prize (Akademie der Wissenschaften zu Göttingen, Germany)
2005:	Philip Levine Lecturer (Rockefeller University)
2006:	Keynote Speaker, Seventh International Congress on Recombinant Antibodies, Boston

2007: Henry Kunkel Lecturer (Johns Hopkins University)y
2007: Keynote Speaker, Keystone Meeting on Biology of B cells in Health and Disease
2008 Keynote Speaker, International Antibody Engineering Conference, San Diego

BOARD MEMBERSHIPS

Academic

1988: Member of Scientific Committee of MRC Clinical Research Centre
1992-1997: Member Advisory Board San Raffaele Basic Research Department, Milan
1997 -2008 Member, Scientific Advisory Board of the Institute d'Immunologie (CNRS), Marseilles, France
1997-2001: Member, Scientific Advisory Board of the Max Planck Institute for Immunology, Freiburg, Germany
2001 -2009 Trustee, Isaac Newton Trust

Service on various committees of the Royal Society, EMBO, MRC, Trinity College, University of Cambridge etc.

Editorial
Previously member of Executive Committee of *European Journal of Immunology* (till 1998), transmitting editor of *International Immunology* (till 2001) and member of editorial boards of *Current Biology* (till 2007) and *EMBO Journal* (till 2009). Currently on editorial boards *Immunity* and *Journal of Experimental Medicine.*

Company
Have consulted for various companies interested in antibody engineering and have served at various times on the scientific advisory boards of Cambridge Antibody Technology, Xenova-Cantab and Therapeutic Human Polyclonals. Currently on the scientific advisory boards of ProteinLogic, Anaptys and OMT. Inventor on various granted patents and applications in the field of antibody engineering